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MECHANISM OF RETARDED MENTAL DEVELOPMENT IN MSP-INDUCED SFD RATS, ESPECIALLY ON DNA SYNTHETIC RATE OF THE BRAIN

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Synopsis Development of mental or behavioral activity after birth of small for date (SFD) rats produced by injection of microspheres at the 18th day of gestation was studied, and obtained the following results: 1. Brain weight of the SFD group increased at a lower level than the control group, but there was significant difference in the brain/body weight ratio between SFD and the control; the SFD group retained a higher value than the control. 2. The peak of brain DNA synthetic rate which measured by intraperitoneal (i.p.) injection of H^3-thymidine was shown at the 10th day after birth in the control group, but it was delayed four days in the SFD group. 3. The maximum incorporation of H^3-thymidine into brain cells was studied by autoradiography. The rate was corresponded to the peak of brain DNA synthetic rate. 4. Content of cytochrome c in the brain mitochondria also increased in higher level than the control group, but there was no difference in the content of cytochrome a between both SFD and the control group.

From these results we discussed the biochemical basis of the process of mental development of SFD after birth.

Introduction

From the clinical and social aspects of view, the development of small for date (SFD) is an important problem after birth. The mechanism of SFD formation is too complex and still little is known about it. However, if SFD could be prepared by experimental conditions, it would contribute to clarify the mechanisms to some extent.

In the previous publications (Moriyama et al., 1976a[8]), Yamaguchi et al., 1976[16]), the authors have found that injection of microspheres (Msp) at the 18th day of gestation could produce SFD in the pregnant rat. Also, in a succeeding paper (Moriyama et al., 1977[10]), Ueda, 1977[15]) we noted that SFD rat could attain the normal level with some delay after birth. In addition, the mechanisms of SFD formation had been reviewed with respect to the placental functions by Moriyama (1976b[9]).

On the other hand, the brain which is unable to repair or to reproduce is most important to mental development as well as somatic development. It is interesting to investigate the functional development of SFD after birth. Shimada et al. (1976[13]) have studied that the newborn rat fed with a low calory diet during pregnancy has been found to be less in glial cell proliferation than the control. In this paper, we focussed out attention on the development of SFD rat brain which was treated with Msp during pregnancy, and studied the mental development of SFD with biochemical aspects after birth.

Materials and Methods

1. Preparation of SFD rat

The preparation of SFD rat has been described in previous publications (Moriyama et al., 1976a[8]), Yamaguchi et al., 1976[16]). The rats after injection of microspheres at the 18th day of gestation, they were observed until spontaneous delivery. After birth, the newborn rats were subjected physiological test and biochemical analysis at the indicated day.

2. Estimation of brain DNA synthetic rate

1 μCi/g of body weight of H^3-thymidine was injected intraperitoneally into the newborn rats. After 60 min., they were sarcified and brain DNA was extracted by the method of Marmur (1961[6]) modified by Moriyama (1974[7]).

3. Measurement of brain mitochondrial

Fig. 1. Growth curve of brain weight of the control and SFD rat treated at the 18th day of gestation with Msp (Mean±S.D.).

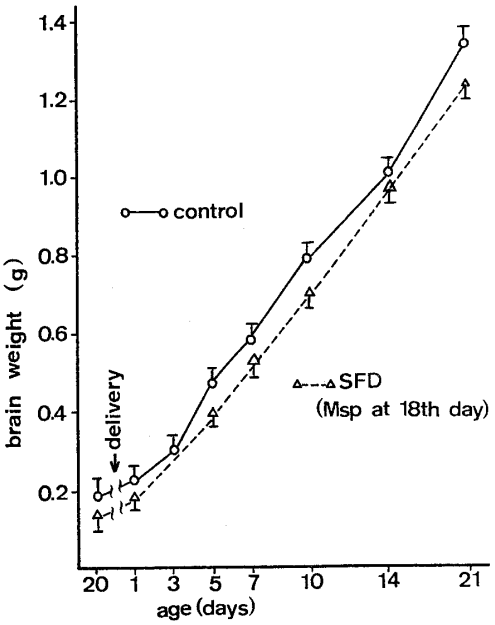


Fig 2. Changes in the ratio of brain weight to body weight after brith (Mean±S.D.).
Msp was injected at the 18th day of gestation.

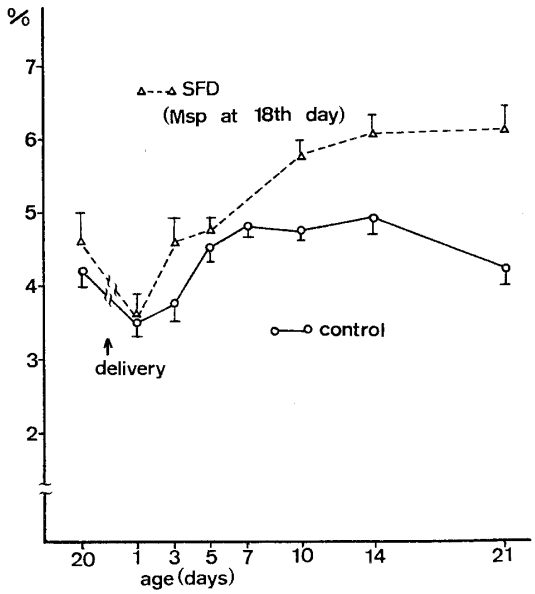
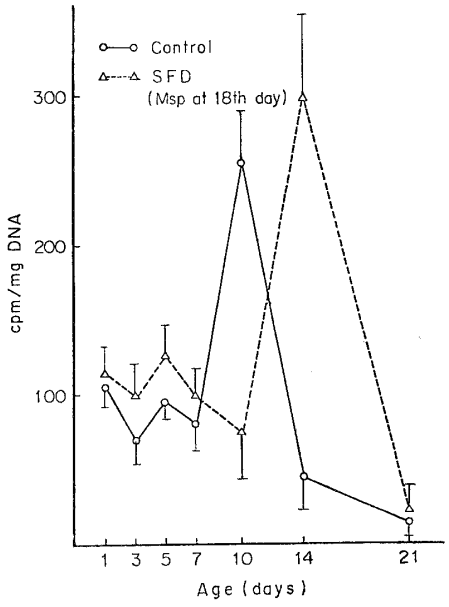


Fig. 3 DNA synthetic rate of the newborn rat brain after birth.
SFD rat was prepared by injection of Msp at the 18th day of gestation. Each curve represents the mean for four or five rats (Mean±S.D.).



cytochrome

After decaputation of newborn rat, the brain was quickly removed, and brain mitochondria was prepared by the method of Hogeboom (1955[5]). The content of cytochrome c and a were measured by the two beam electric photometer (Shimazu, Co. Ltd.), respectively.

4. Autoradiography

1 μCi/g of body weight of H^3-thymidine was injected intraperitoneally. 60 min. later, the rat brain was removed and fixed into 10% formalin. After preparation, the brain section was admitted to the dipping method with Kodak-NTB-2 adsorption for two weeks and then stained with hematoxylin-eosin.

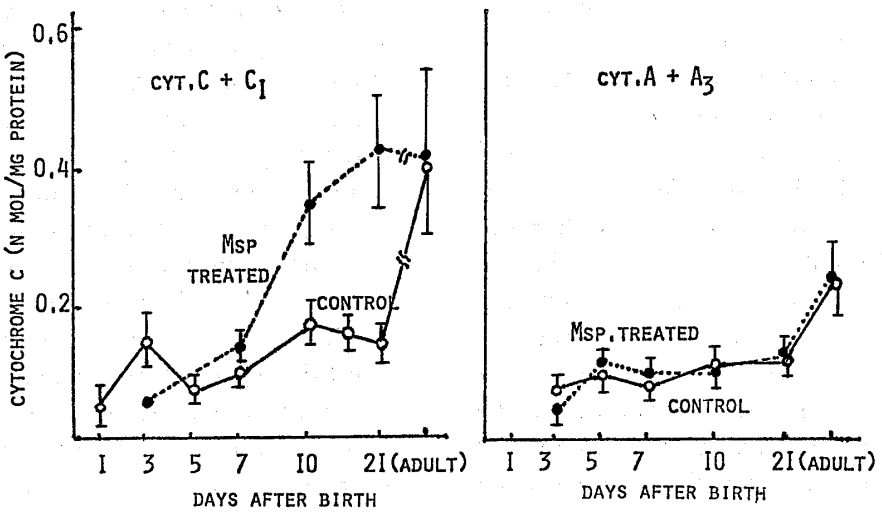
Results

I. *Postnatal development of SFD rat brain*

SFD rat was prepared by injection of Msp at the 18th day of gestation as described previously (Yamaguchi et al. 1976[16]). After delivery, the brain wet weight of SFD rat was measured at the scheduled days for three weeks. The results are shown in Fig. 1.

As the results are shown in the figure, the brain weight of SFD rat increased at a lower level than the control. At the end of the third week, it was still about 14% lower than the

Fig. 4. Cytochrome content of brain mitochondria in newborn rat derived from the dams treated with Msp at the 18th day of gestation (Mean±S.D.).



control. To clarify more precisely, we estimated the brain/body weight ratio from the above-described result. The results are shown in Fig. 2. It was clearly demonstrated that the brain/body weight ratio showed a reverse pattern in each period during the development after birth. The ratio increased gradually in the SFD group until the second week after birth then came to a steady state. The ratio of the SFD group was in a higher value than the control during three weeks after birth.

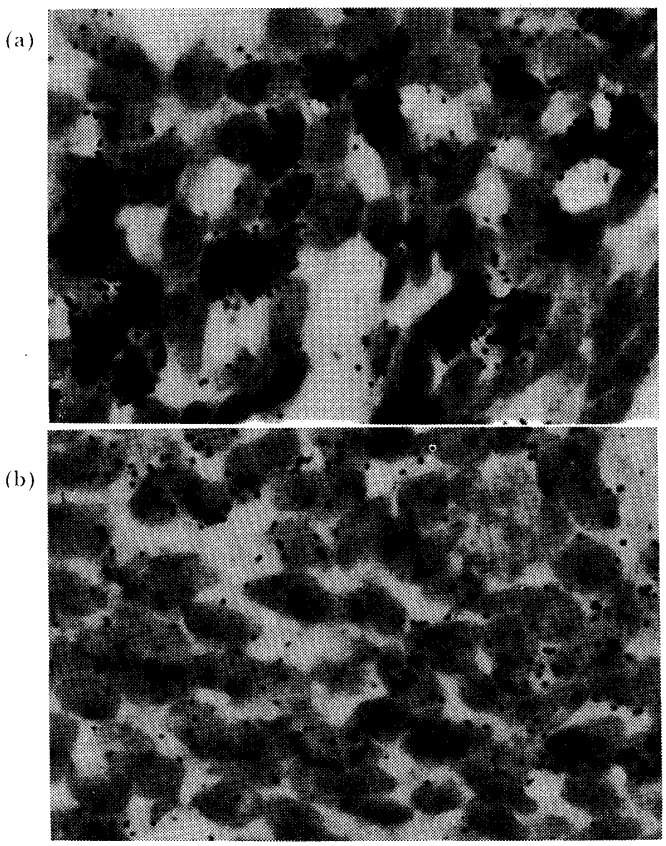
II. *Brain DNA synthetic rate of SFD rat during the development*

In the previous paper (Moriyama et al., 1977[10]), we have seen that the behavioral and mental development of SFD rat was delayed with some latency. This means that the in the SFD brain organization in the SFD may be more incomplete than the control. In other words, the proliferation of brain cells may be delayed in the SFD group.

To check the problem, brain DNA synthetic rate was measured in both SFD and the control group after intraperitoneal injection of H^3-thymidine. DNA synthetic rate was expressed in cpm/mg of brain DNA fractions after birth.

As shown in Fig. 3, DNA synthetic rate attained the maximum on the 11th day after birth in the control group. On the contrary, it attained the maximum on the 14th day in the

Fig. 5. Autoradiography of the newborn rat brain. H^3-thymidine was injected ip 1 hours prior to sacrifice at the 14th day of gestation. Upper figure (Fig. 5a) shows the autoradiography of Msp treated rat brain at the 18th day of gestation and lower figure (Fig. 5b) shows the control rat brain. Both figures show the autography at the 14th day after birth.



SFD group. The maximum rate was delayed for four days in the SFD group. In spite of low weight in SFD brain, the DNA synthetic rate kept a higher level than the control.

III. *Cytochrome content of brain mitochondria during the development*

In the above experiment, brain DNA synthetic rate in the SFD had been kept at a higher level than the control. This means that the metabolic activities of SFD brain may be higher than the control. It may be possible to consider that such metabolic energy source may be introduced through the oxidative phosphorylation of brain mitochondria. To confirm this, we estimated the metabolic activity of brain mitochondria by measuring the mitochondrial cytochrome content.

Mitochondrial fractions of newborn rat brain from both groups were prepared, and the cytochrome contents were estimated as the forms of cytochrome $c+c_1$ and cytochrome $a+a_3$ respectively from the second week after birth. There was significant difference between SFD and the control group in the content of cytochrome $c+c_1$ until the adult stage.

IV. *Autoradiography of SFD brain*

In the former experiment, we found that DNA synthetic rate was different in both groups. To confirm the cellular level incorporation of H^3-thymidine in the brain, after intraperitoneal injection of H^3-thymidine, the autoradiography in the SFD and the control was compared at the 14th day after birth.

As shown in Figs. 5a and 5b, it was observed that the SFD brain was stained more densely than the control at the same 14th day. In Fig. 5a, many immature cells which seemed to be megaloblastocytes were also observed. In the control, however, the same results were observed at the 10th day after birth. These phenomena agreed with the previous result in which the maximum of DNA synthetic rate was seen at the 10th day in the control and the 14th day in the SFD group after birth, respectively.

Discussion

Mental development of SFD was studied with reference to brain weight and behavioral activity. In addition, DNA synthetic rate and other biochemical changes were also studied. Brain weight of SFD increased at a lower level than the control, but the brain/boby weight ratio of SFD and of the control showed quite different patterns; the ratio of SFD continued a higher value than the control for three weeks during the observation period. This suggests that the brain in SFD rat is more preferentially protected than the other organs.

Mental development of SFD measured by the conditioned avoidance experiment could reach the normal level after a delay of several days. Hess (1953[4]) and Aizawa (1963[1]) have stated that SFD could develop more rapidly than the premature baby in human beings. On the other hand, Roux (1971[12]), Fitzhardinge (1972[2]) and Smart (1974[15]) have stated that mental activity and brain development in SFD rat could not attain the normal level.

From the present experiment, DNA synthetic rate of SFD brain proceeds at a higher level and reaches the peak at the 14th day showing a delay of several days from the control. It has been suggested that there was a delay in attaining the peak of DNA synthesis in the brain in hyper- or hypothyroidism rat (Nicholson, 1972[11], Hamberg, 1971[3]). Also, the present autoradiographic experiment showed that H^3-thymidine was more densely incorporated into the SFD brain stem than into that of the control at the 14th day after birth. The reverse phenomenon could be seen at the 11th day after birth. This agreed with the chemical data of DNA synthetic rate in the SFD brain. Cytochrome content also retained a higher level than the control until the third week. It is possible to speculate that the metabolic rate may be accelerated in SFD brain to compensate for the delay of development.

The data presented here and our previous report strongly suggest that delayed cell development in the brain in the fetal period may cause a long-period delay of mental development of the offsprings although it can

finally attain the normal levels.

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